EuroBiofilms Workshop 3 - Molecular diagnostics of biofilm bacteria

Microfluidic Systems and Confocal Microscopy.

FLOW CELL SYSTEMS

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Workshop Objectives

- Introduction to importance of shear and biofilm accumulation
- Identify experimental objectives
- Hydrodynamic parameters relevant to biofilms
- Selection of biofilm flow cells
- Growth systems

Biofilms – Wide Range of Shear Stresses

Marine Biofouling

- Hulls
- Structures

Host tissue

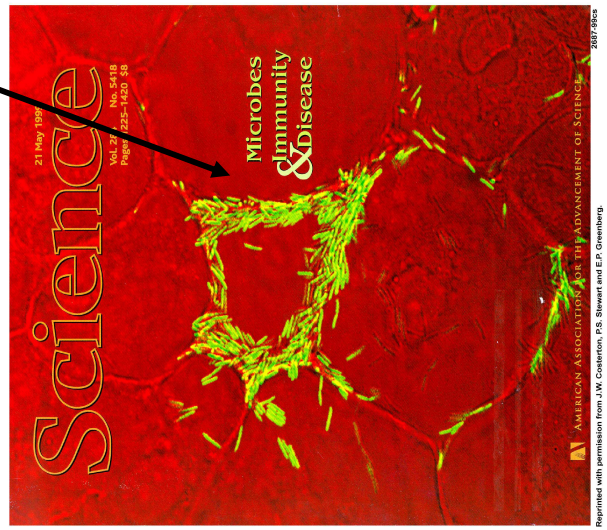
- Infective endocarditis
- CF lung

Nosocomial

- Catheters
- Dental water lines

Public Health

- Drinking water



Biofilms in Industry

Problems

Product contamination

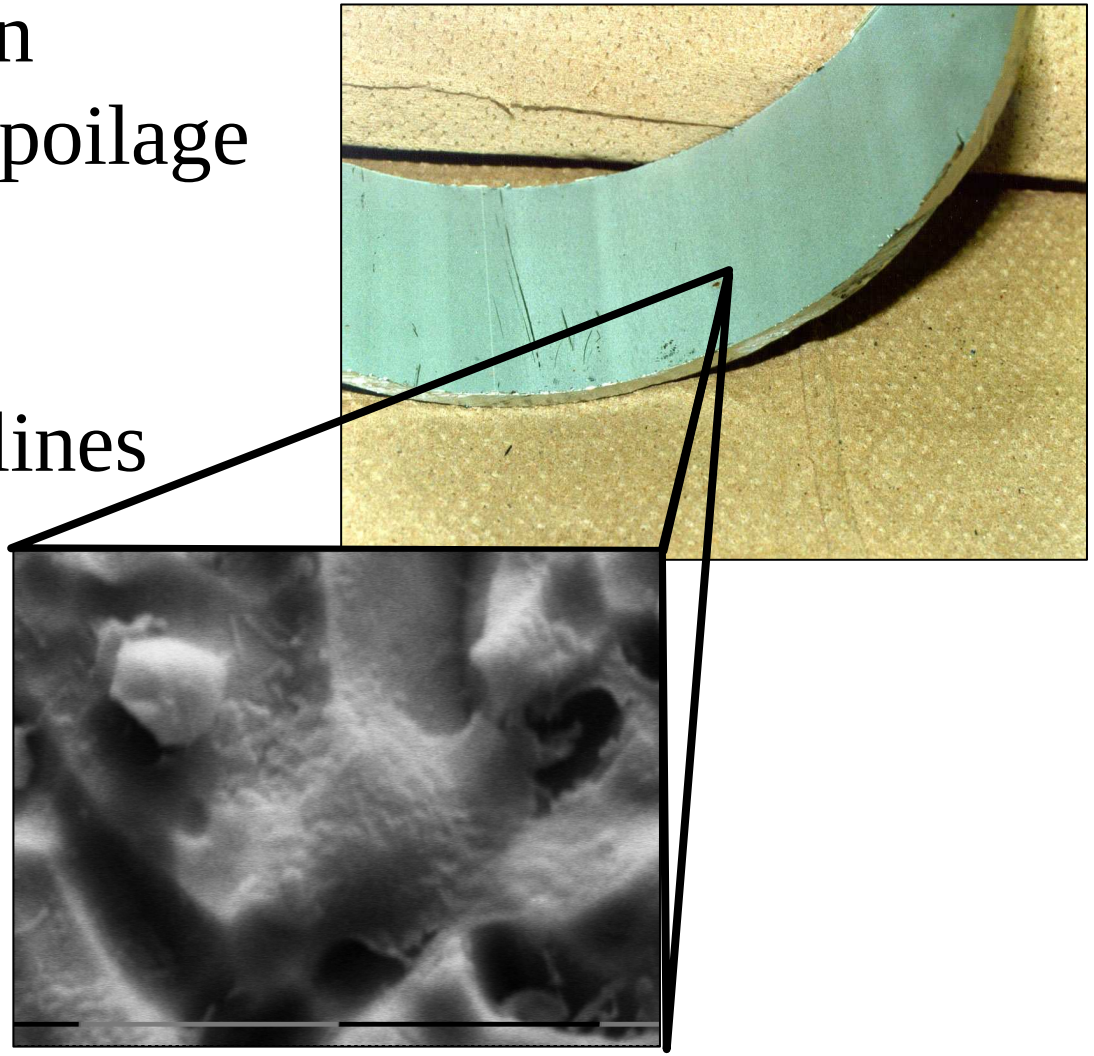
Public health / spoilage

Membrane plugging

Corrosion

Energy losses in pipelines

SRB oil souring

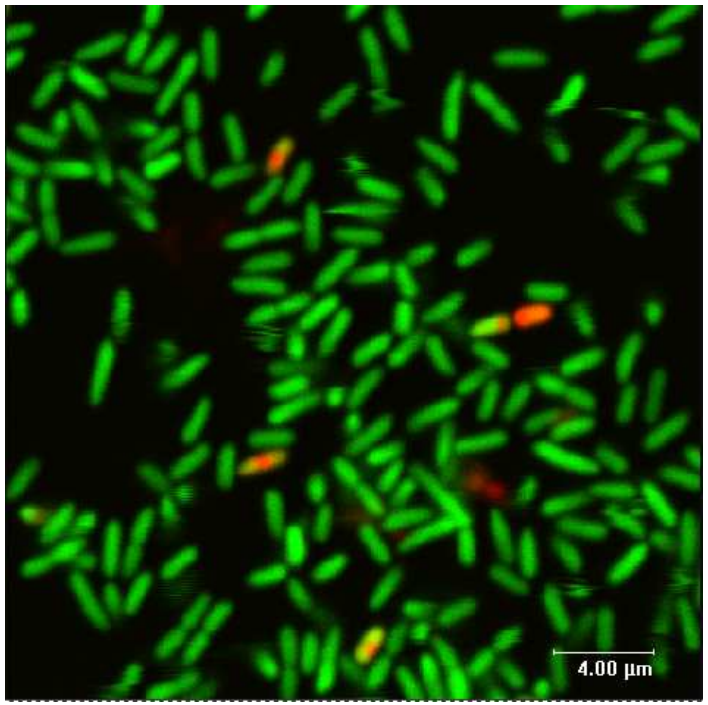


Experimental Systems for Quantifying Biofilm Attachment, Accumulation and Detachment in Flow

Experimental Design

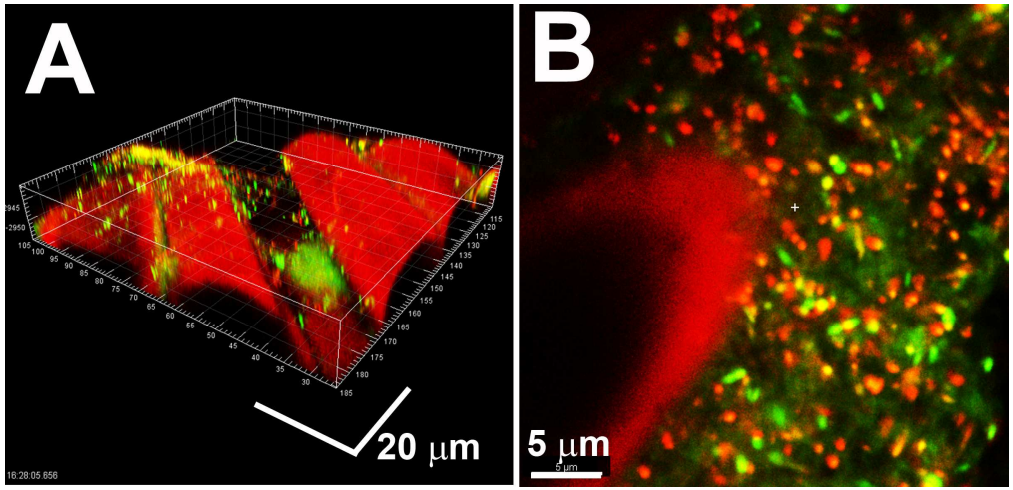
- Identify Relevant Parameters

Laboratory World



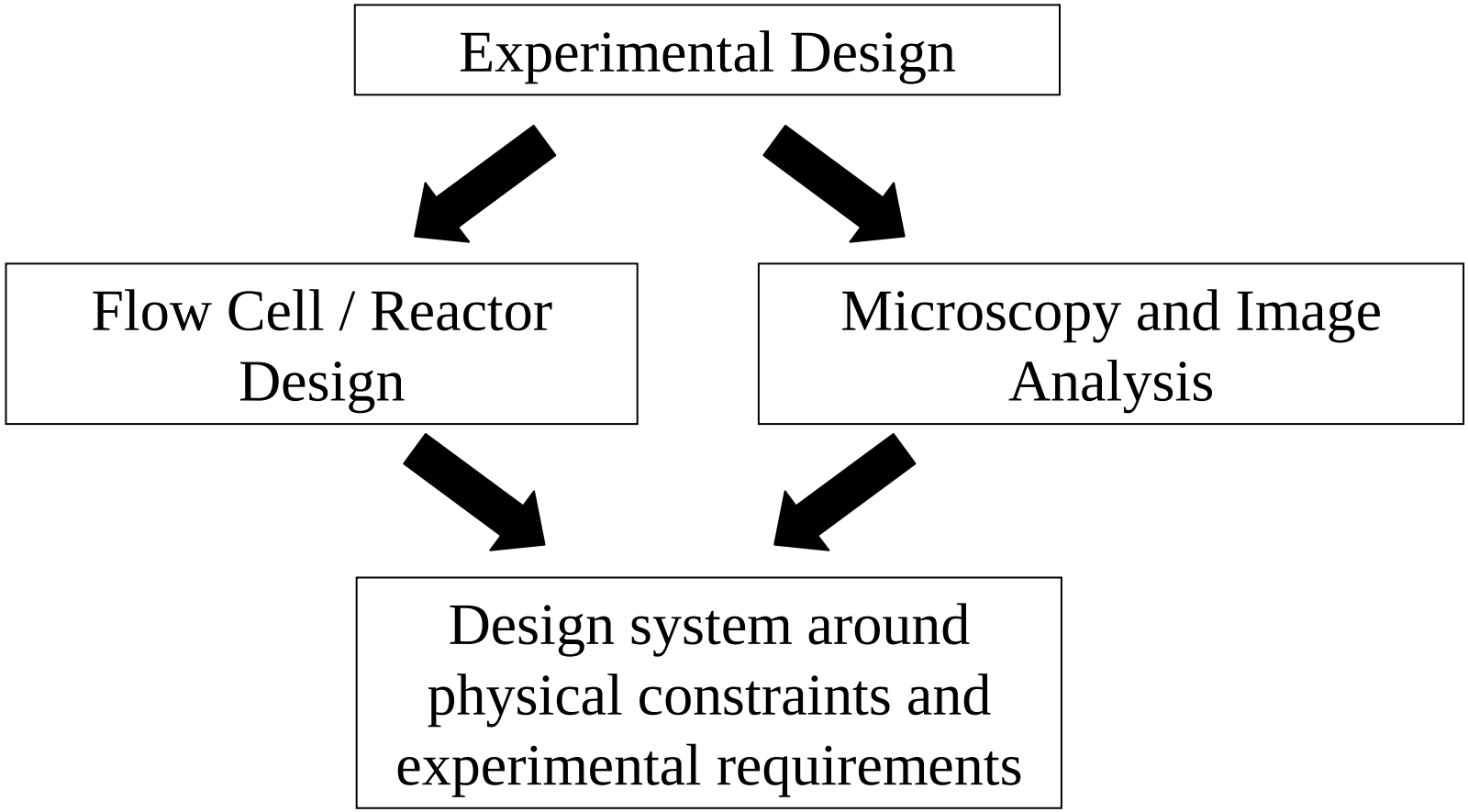
Highly controlled
Investigate factors in isolation
Relevance?

Real World



Highly relevant
Little control
Interpretation?

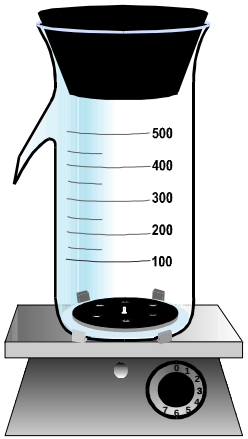
Imaging Biofilms

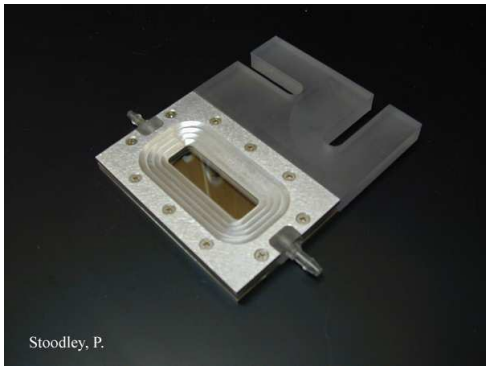


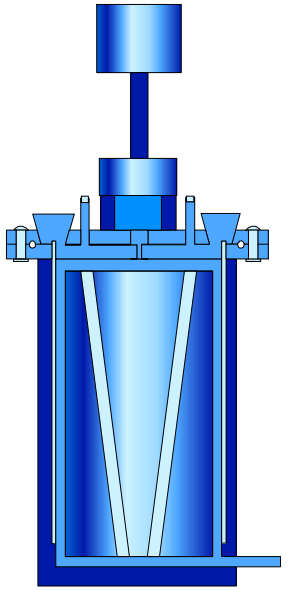
1st Choice: Emphasis on Imaging or Sampling

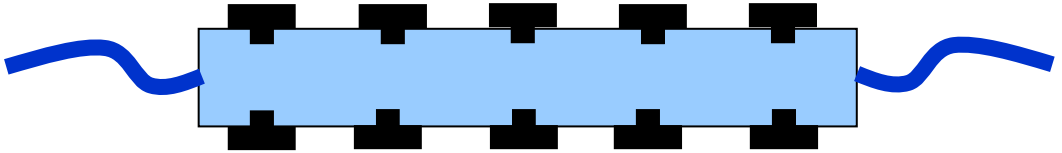
Imaging: 2 basic systems “once through” or “recirculating”

Sampling: Robbins device, annular reactor (rototorque)









Flow Cell / Reactor Design

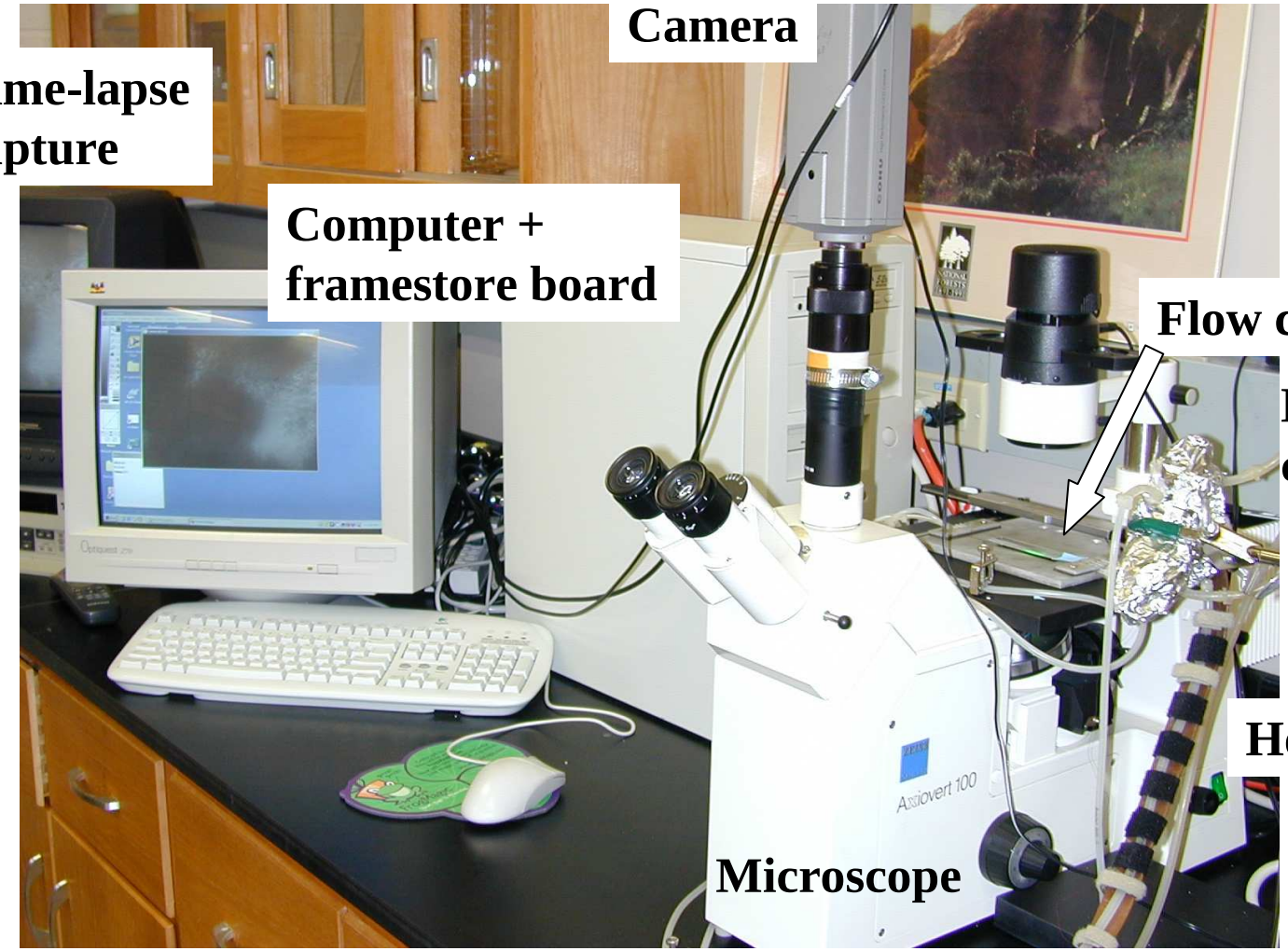
Hydrodynamics

Magnification range – scales of observation
Reactor wall thickness may limit high definition high power microscopy

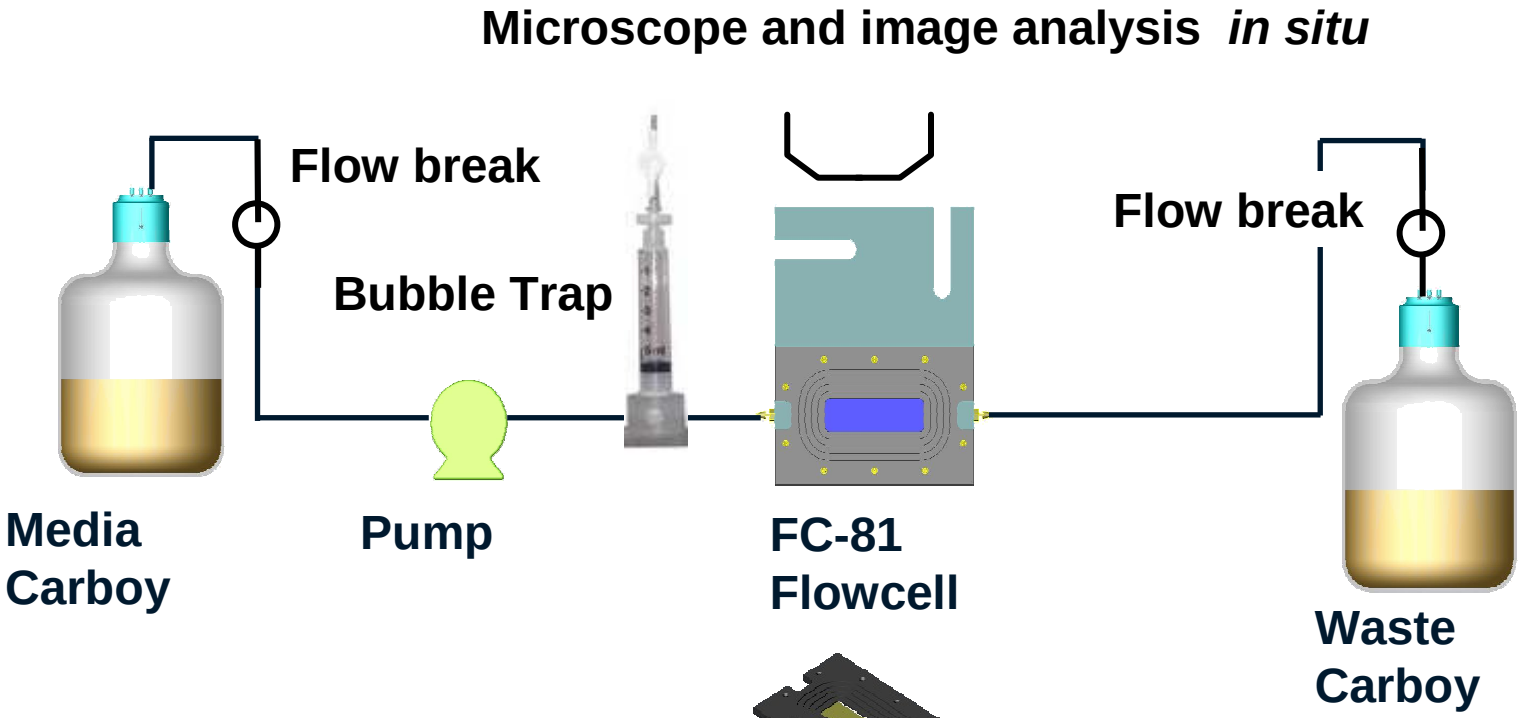
Surface of interest – transparent or opaque?

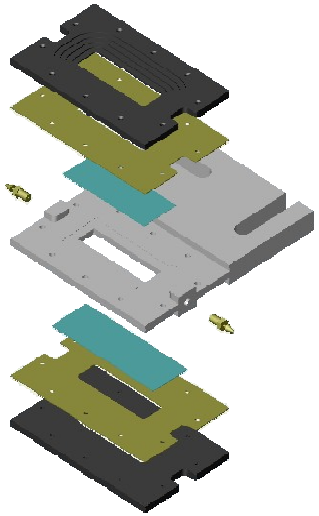
Sampling required?

Experimental System

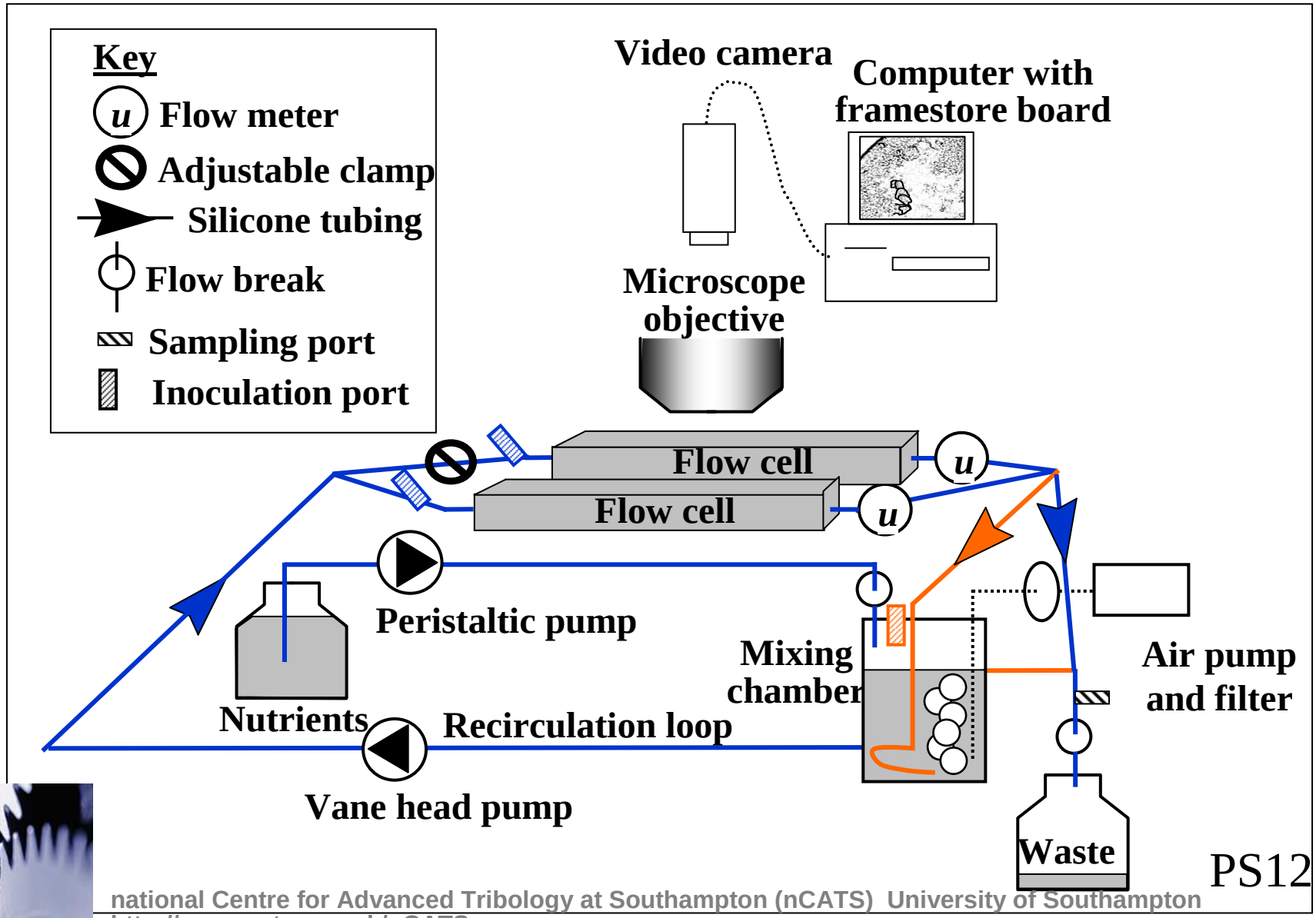


Flow Cells for Biofilm Research

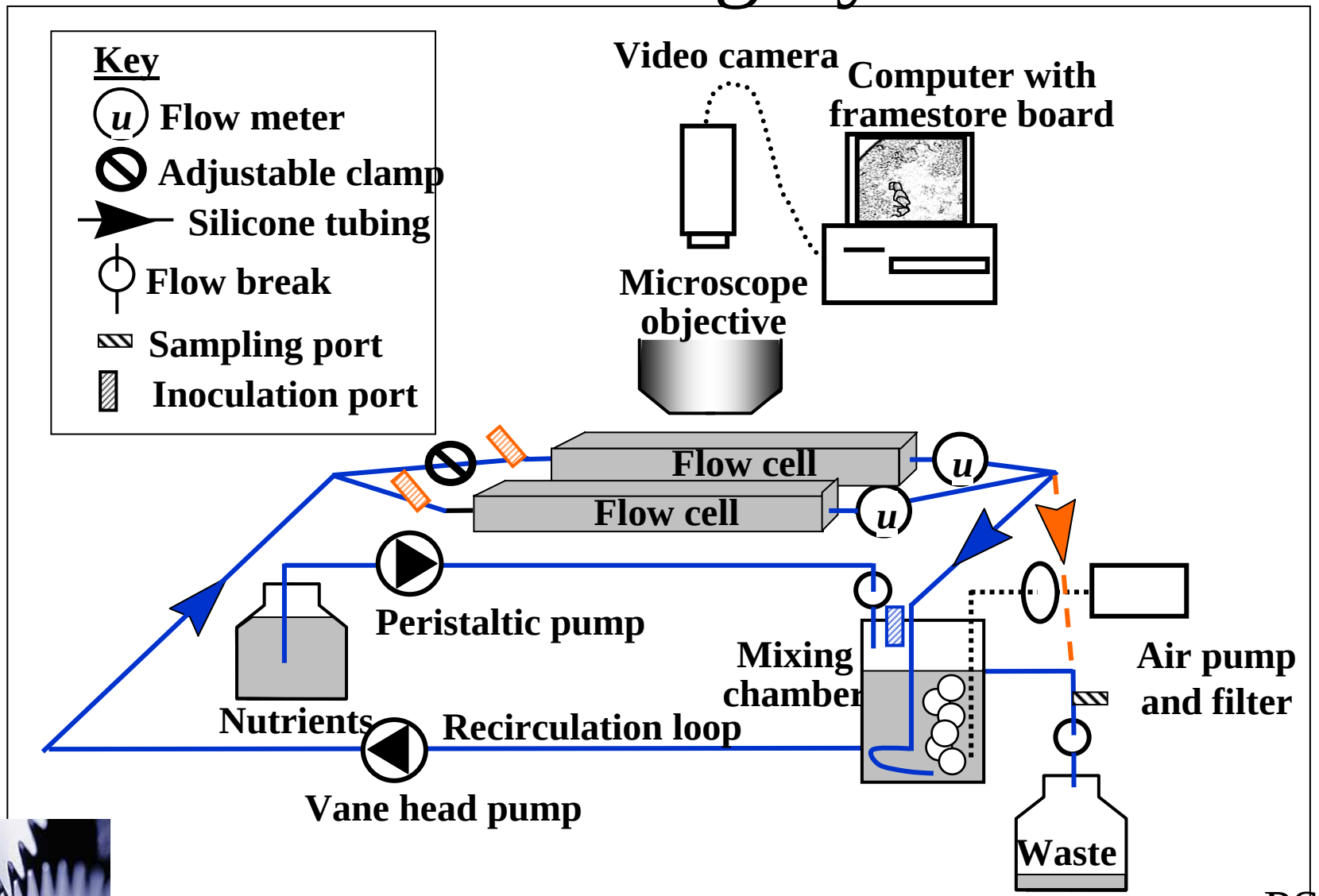




“Once-through” System

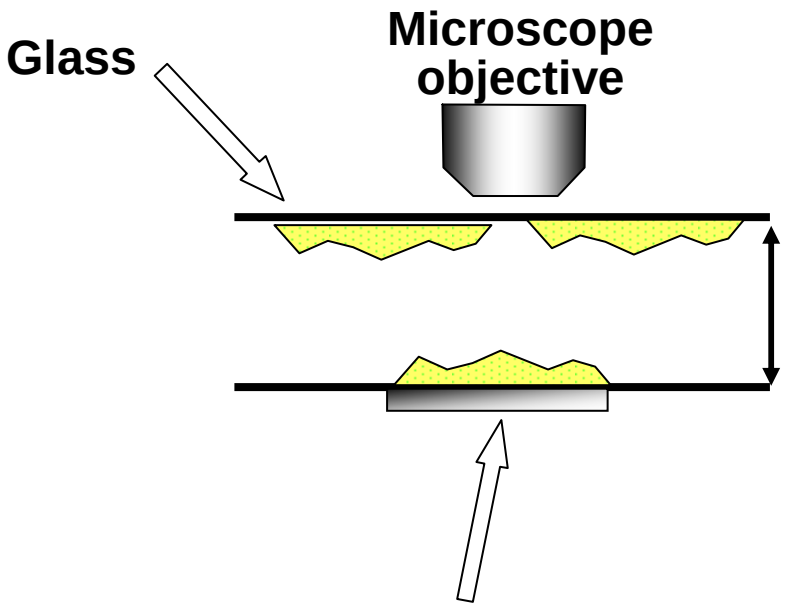


Recirculating System



Flow cell operating requirements

High resolution microscopy – requires flat surface and a transparent window

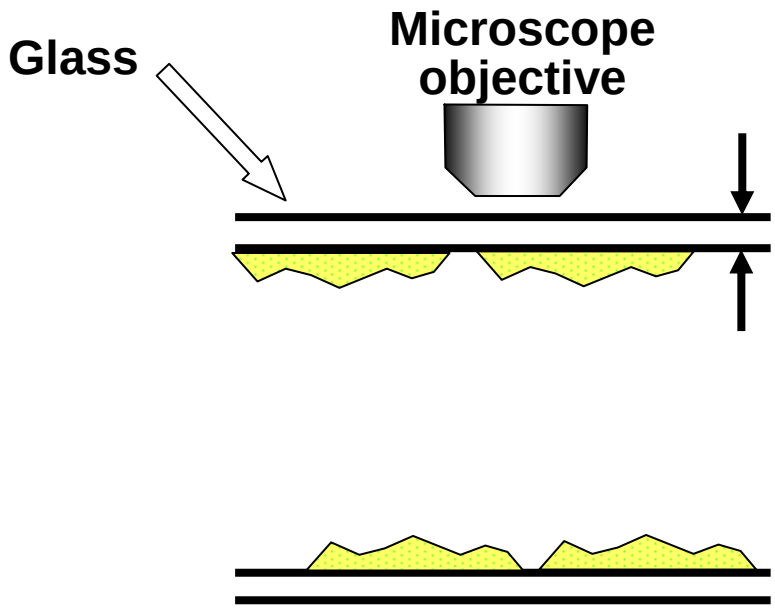


Channel width limited by objective working distance, limits flow rate

Opaque coupon – i.e. steel

Flow cell operating requirements

High resolution microscopy – requires flat surface and a transparent window



Channel wall width limited by objective working distance, limits rigidity

Where to get flow cells

BioSurface Technologies (http://cu.imt.net/~mitbst/flowcell.html)

Fluxion

Stovall (http://www.slscience.com/flowcell.html)

Make your own

Microscopy

Bright field (phase / interference contrast)

Epi-fluorescent

Reflected (interference contrast)

Confocal (3D fluorescent / transmitted)

SEM/TEM

Stains

DNA - AO, PI, EB, SYBR Green

Activity - CTC

Viability - Live dead

Species identification - FISH (16S RNA)

Gene expression - GFP transcriptional or constitutive reporters

EPS - Lectins

Proteins

Check out Molecular Probes http://www.probes.com/

Free Image Analysis Packages

NIH Image (Mac) Image (Mac or PC)
rsb.info.nih.gov/nih-image/
(also lists commercial packages)

Scion Image (PC version of NIH image)
www.scioncorp.com/

ImageTool (PC)
macorb.uthscsa.edu/dig/itdesc.html

What can we measure?

Microscopically

Surface area coverage

Thickness

Length dimensions of biofilm structures

Count objects

Brightness

Rates of change

Molecular tools - GFP expression

Material properties

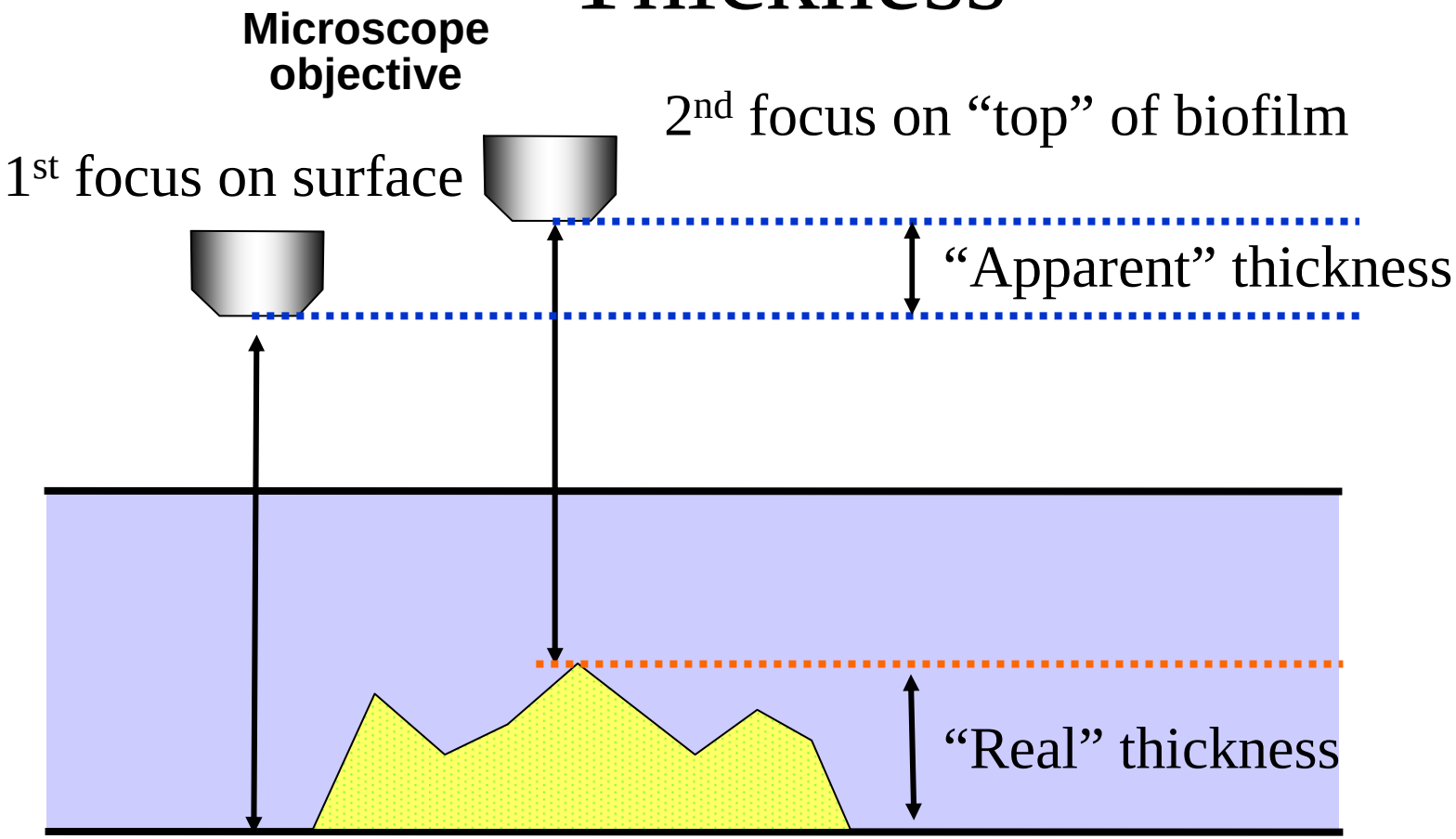
External monitoring

Pressure drop

Effluent cell count

Biofilm cell count (end point)

Thickness



Real thickness = 1.6 x apparent thickness

Recirculating System

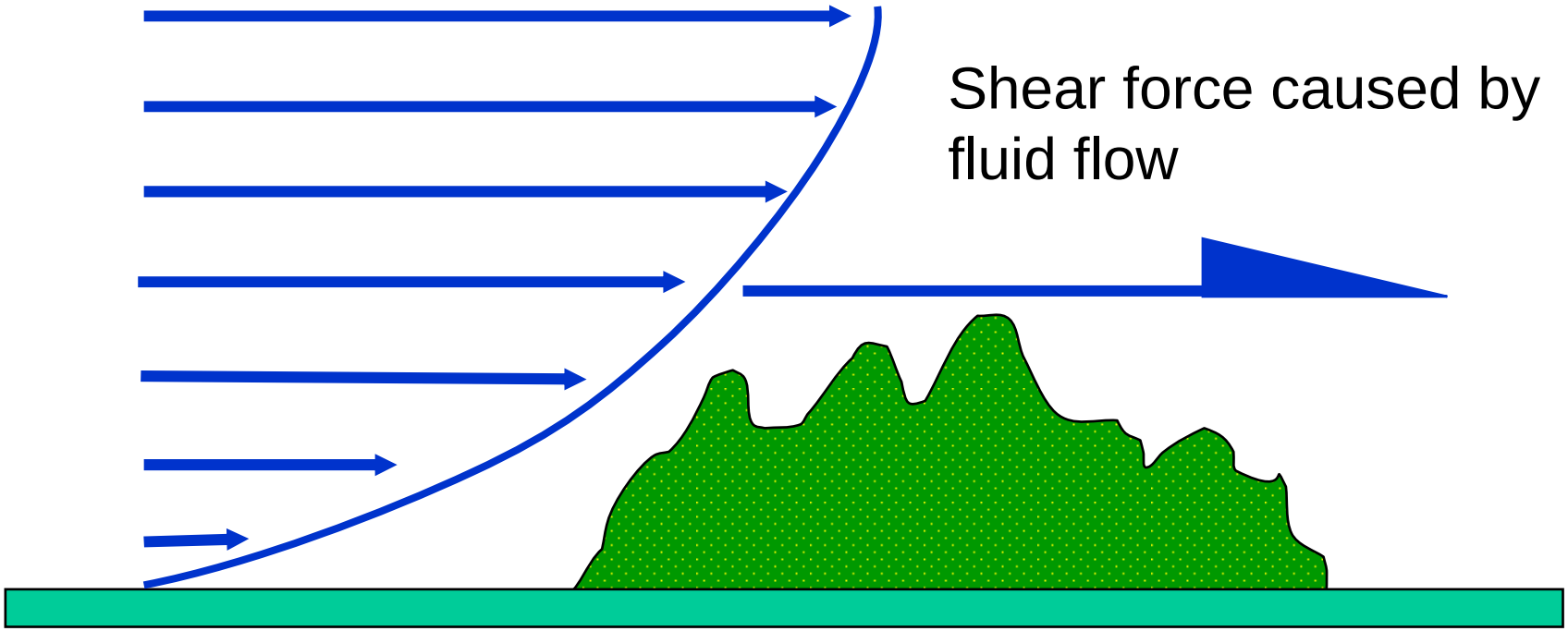
The residence time (θ) can be controlled by the nutrient flow rate (Q_n) according to $\theta = V/Q_n$, where V = the volume of the mixing chamber *plus* the recirculation loop.

The flow rate in the flow cells can be adjusted independently of the nutrient flow rate so that much higher flow rates can be achieved in the flow cells without using impractical volumes of media.

Q_n can be adjusted so no planktonically dividing population by making the dilution rate (D) greater than the growth rate (μ)

$$D = Q_n/V \qquad \mu = \ln(2)/Dt$$

Hydrodynamic characterization



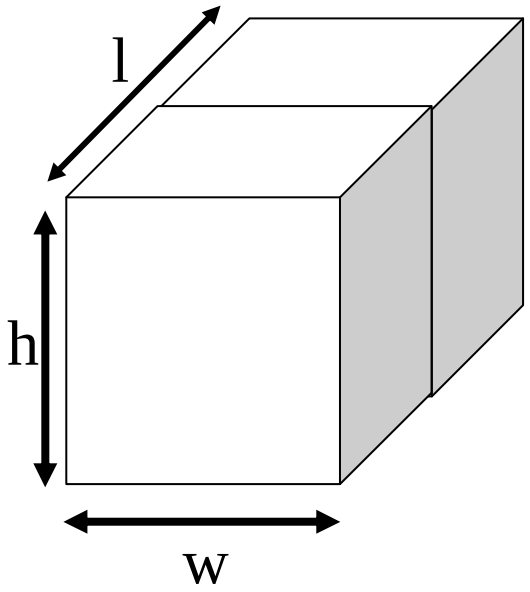
Hydrodynamic Parameters for Closed Channel

Flow rate (Q) ml/min or m^3/s

Flow velocity (u_{ave}) = Q/CSA (m/s)

Channel cross sectional area, CSA = hw (m^2)

Wetted perimeter, WP = 2w + 2h (m)



Reynolds number = $ul_{ch}\rho/\eta$

$L_{ch} = 4D_h = 4CSA/WP$

η absolute viscosity
ρ density
ν kinematic viscosity = η/ρ

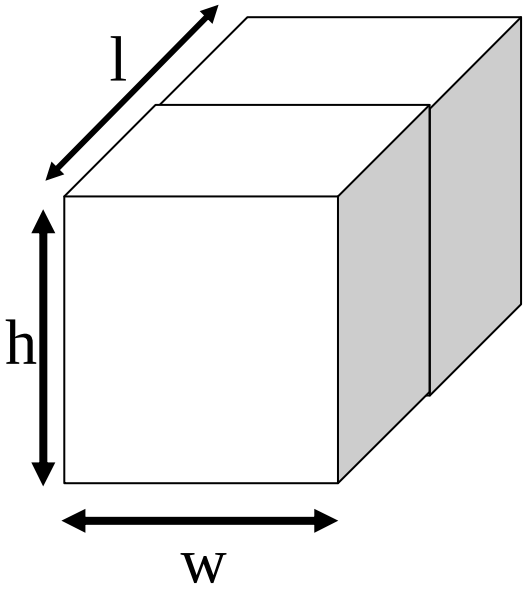
Hydrodynamic Parameters for Closed Channel II

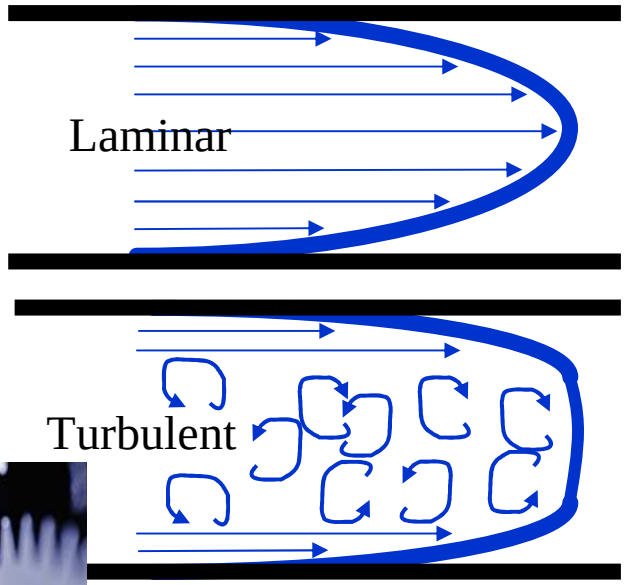
Reynolds number

<500 laminar (low shear)

500 – 1500 transition

> 1500 turbulent (high shear)





η absolute viscosity

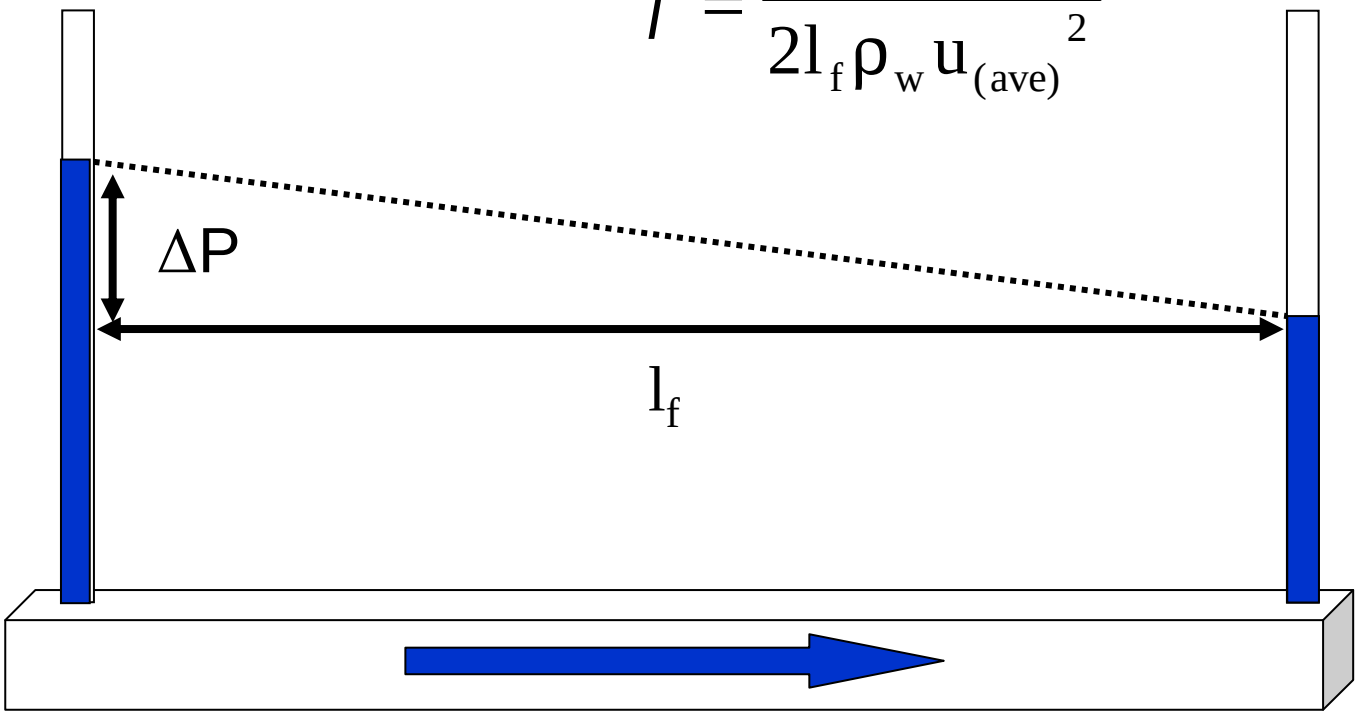
ρ density

ν kinematic viscosity = η/ρ

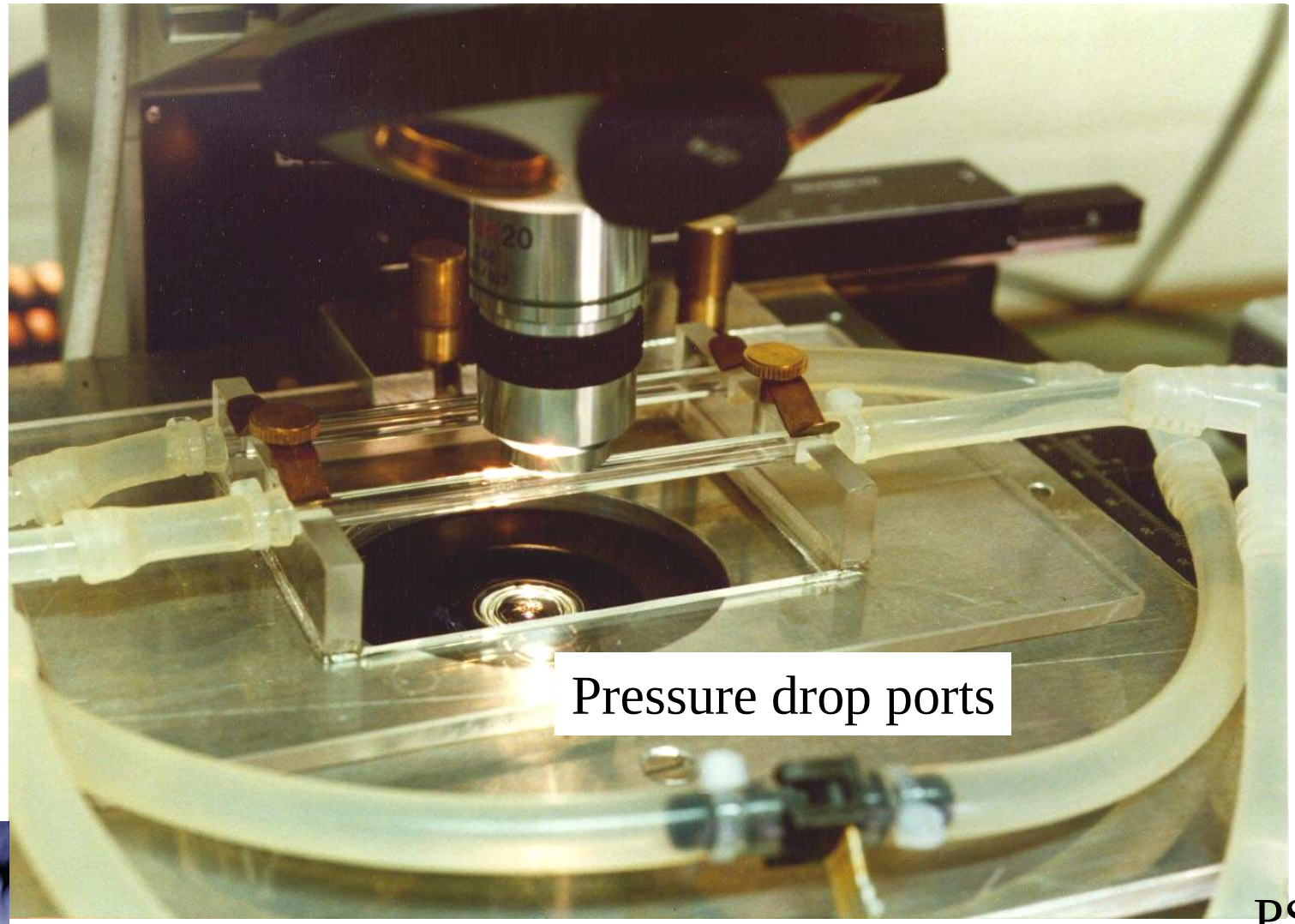
PS25

Pressure drop as fluid moves down a tube due to friction

$$f = \frac{\Delta P \times D_h}{2 l_f \rho_w u_{(ave)}^2}$$



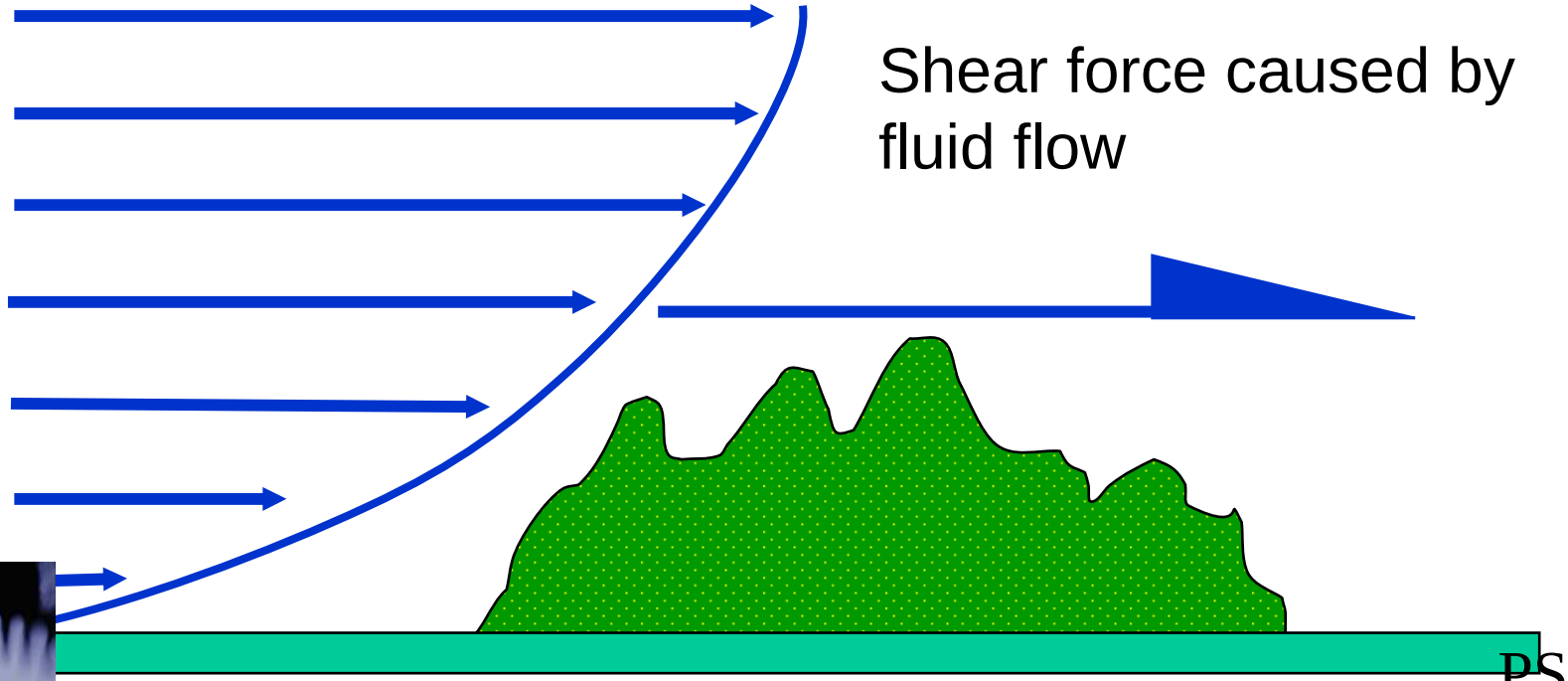
Biofilm Flow Cell Hydrodynamics



Friction factor and shear stress

$$f = \frac{16}{\mathrm{Re}} \quad \text{Laminar flow}$$

$f = 0.0791/\mathrm{Re}^{0.25}$ Blasius equation for turbulent flow



Shear force caused by fluid flow

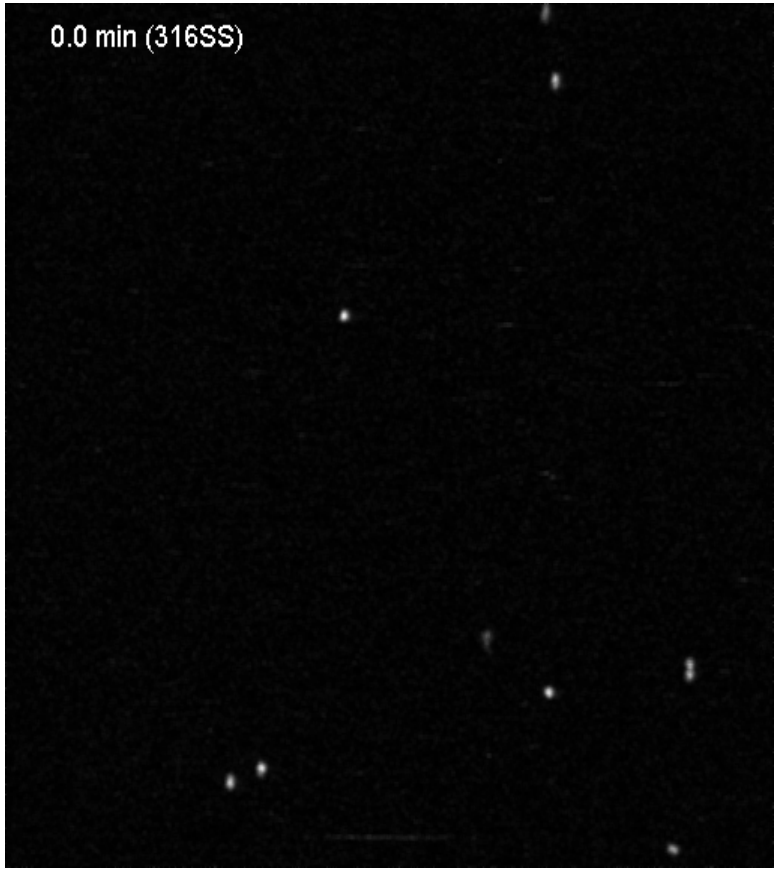
Shear stress

$$\tau_w = \frac{f \rho_w u_{(ave)}^2}{2}$$ Turbulent flow (empirical)

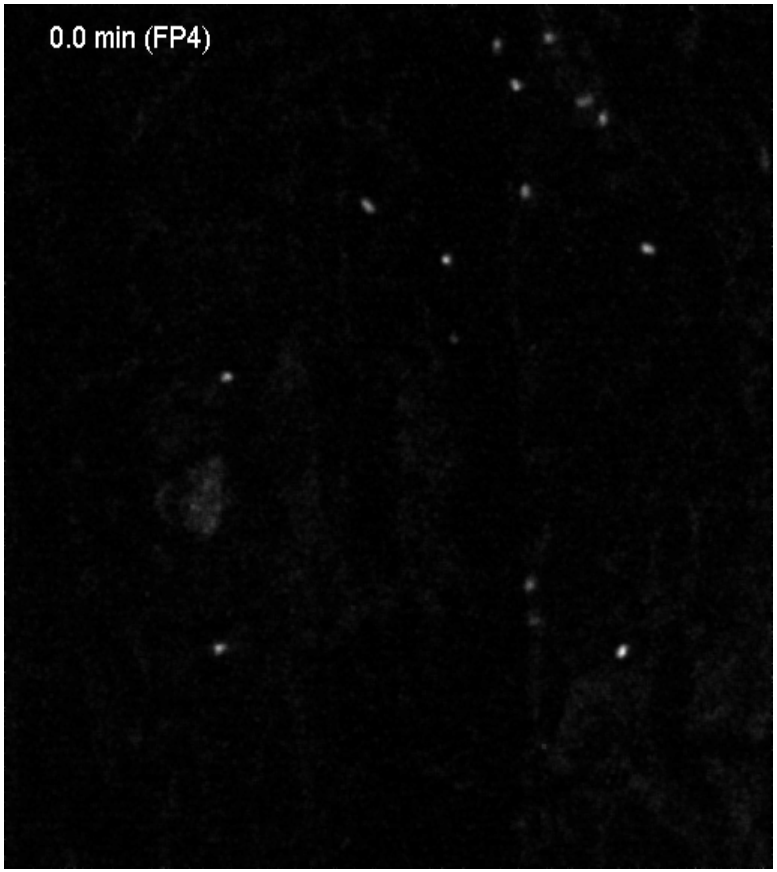
$$\tau_w = \frac{4 \eta u_{(max)}}{D_h}$$ Laminar flow (analytical solution)

Square or circular tube $u_{max} = 2\ u_{ave}$
Flat plate $u_{max} = 3/2\ u_{ave}$
Dh= channel depth

ATTACHMENT ASSAYS



316 Stainless Steel

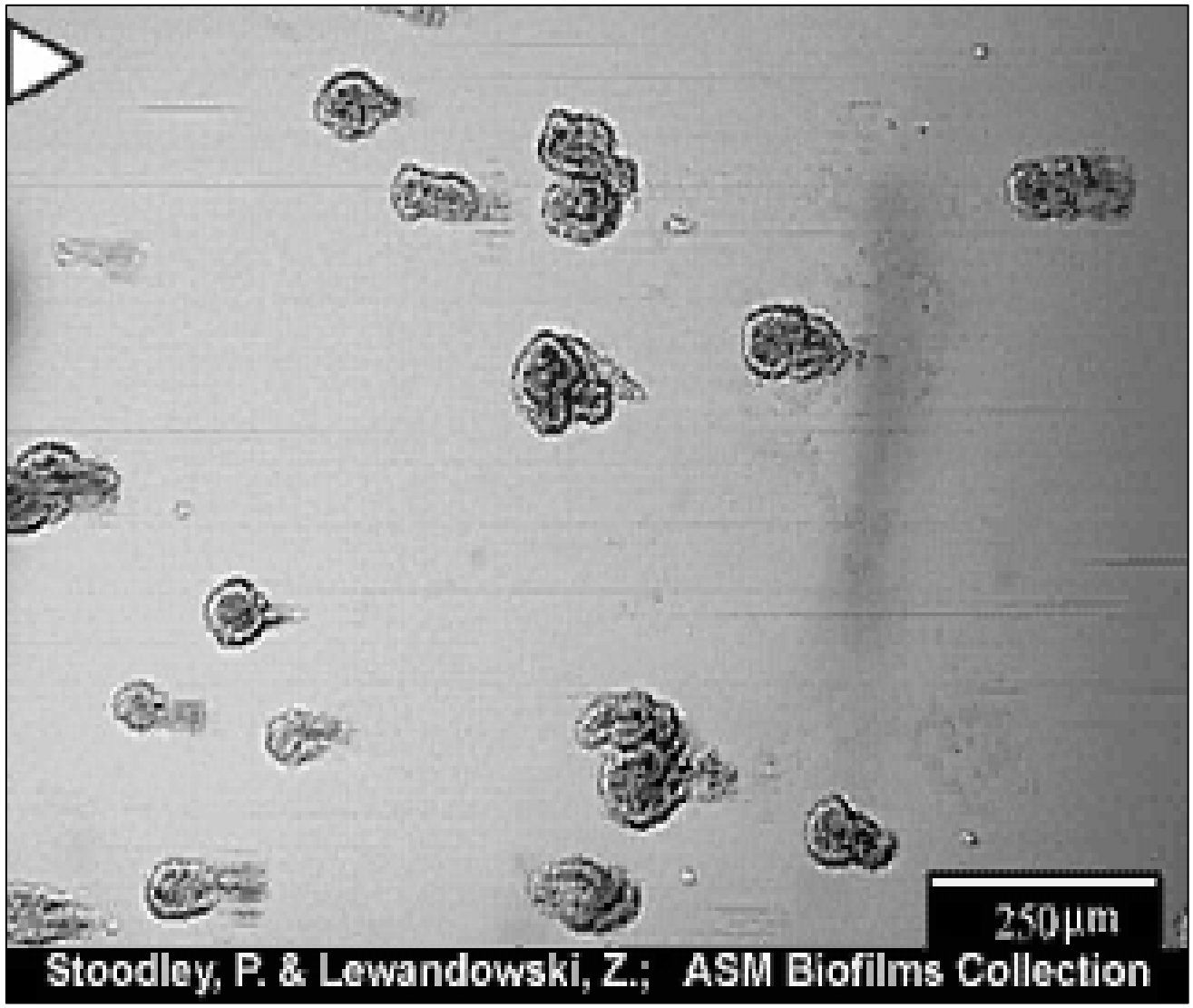


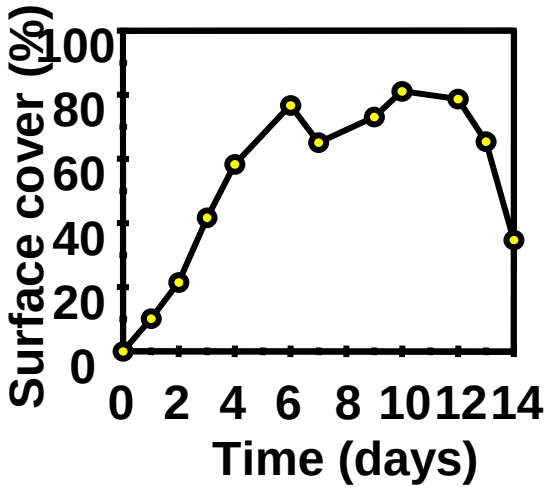
Fluropolymer

PS30

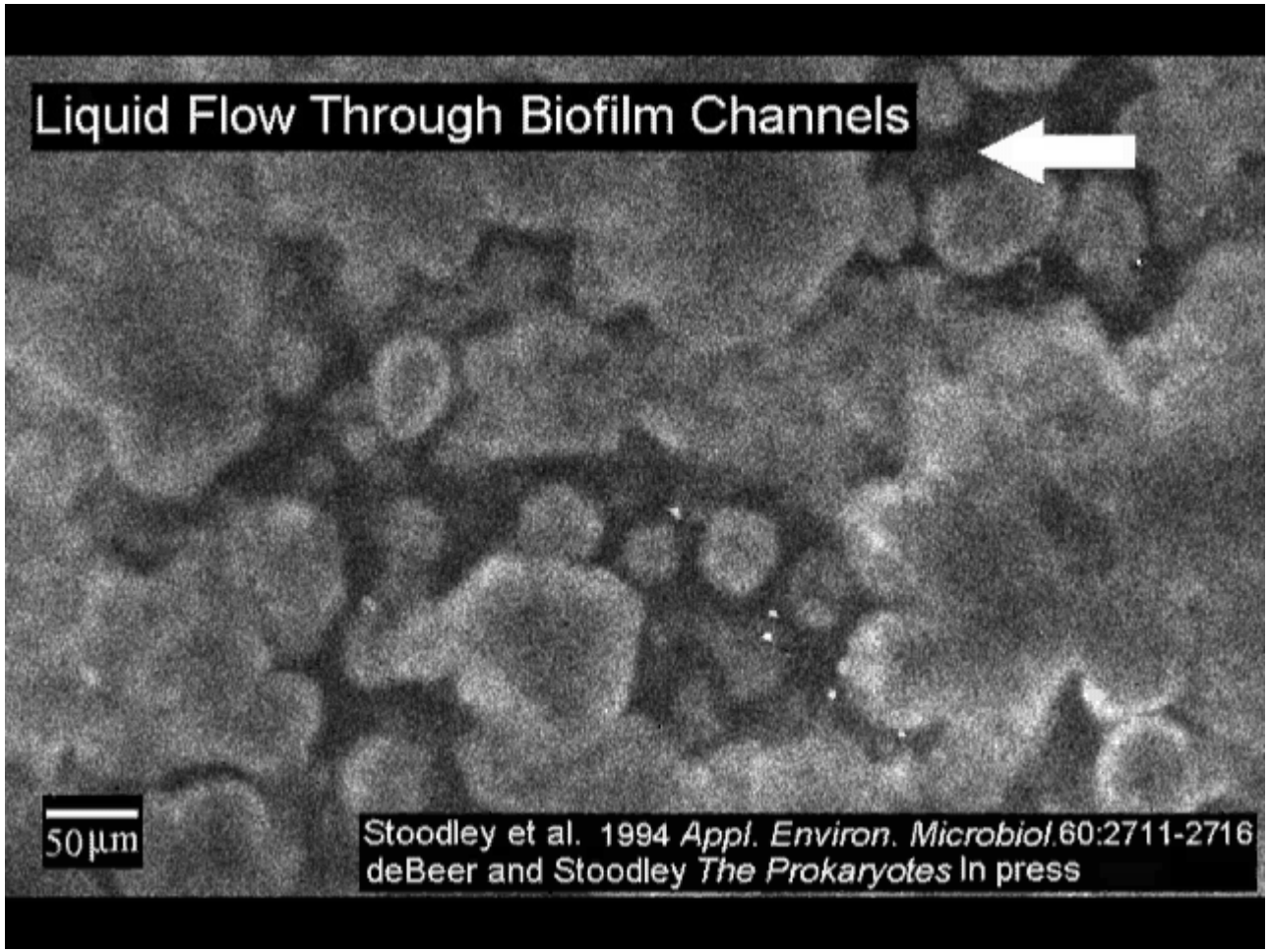
P. aeruginosa **PAO1**

Growth Assays

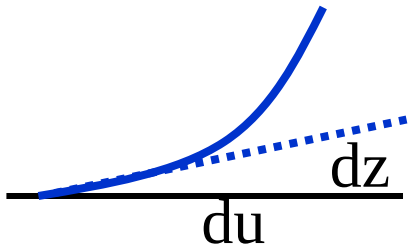




Liquid Flow in Biofilm Channels

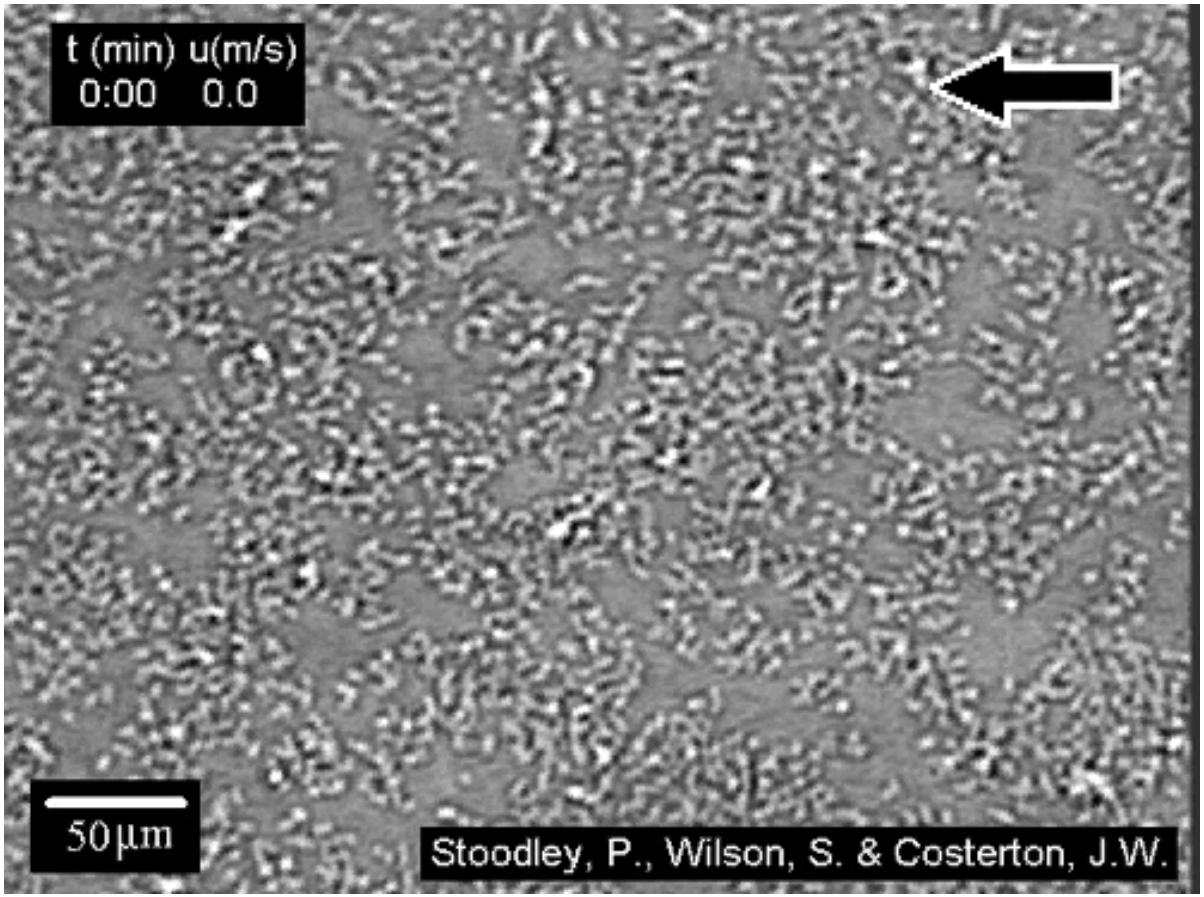


$$\tau_w = \frac{\eta \mathrm{du}}{\mathrm{dz}}$$

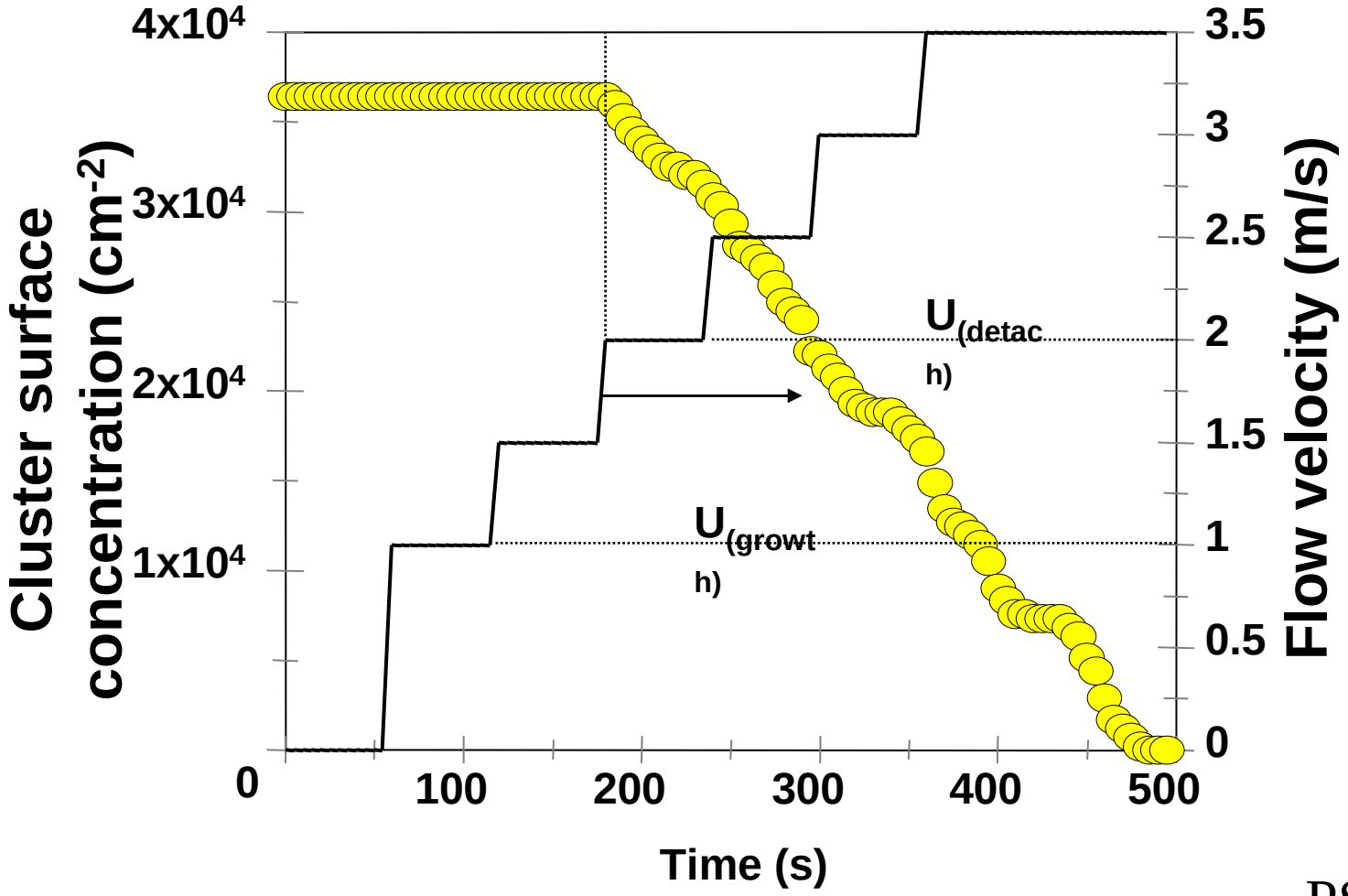


du/dz can be found using particle velocimitry with confocal microscopy

Adhesion Assays

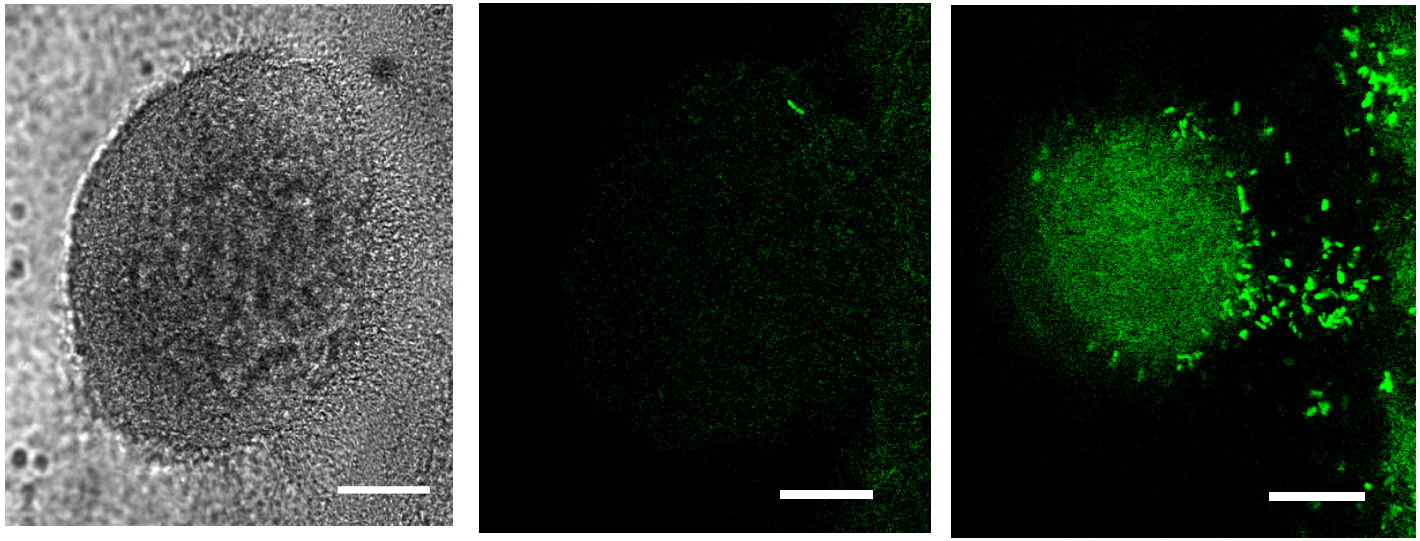


Shear induced detachment



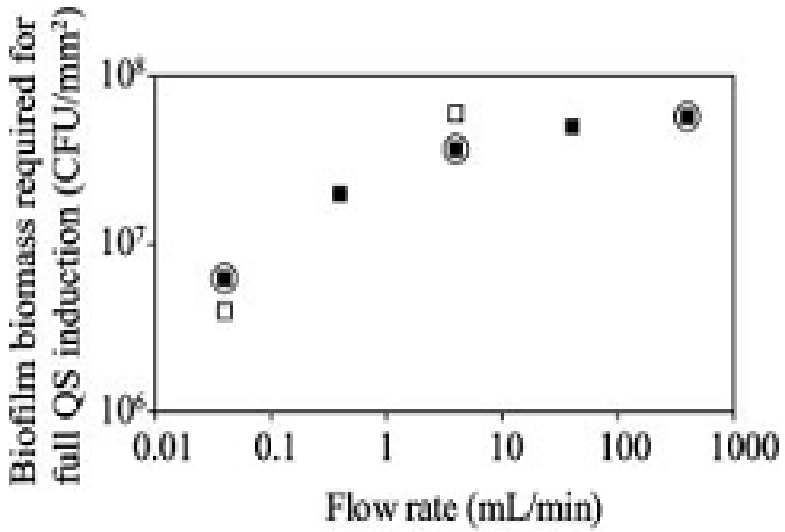
Cell Signaling Experiments

- **Expression of GFP was only observed in the biofilms after flow was turned off.**



P.aeruginosa pMH509 *lasB*::GFP

5-day old biofilm, Q=1ml/min



Thank You to BioSurface Technologies for Supplying Flow Cell Materials

Questions?